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Effect of Auxins with Cytokinins on Stem and Leaf Explants of *JUSTICIA GENDARUSSA* BURM. F.

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ABSTRACT:
The present study describes the effect of combinations of auxin with cytokinin on the callus induction, root induction and direct shoot induction from the stem and leaf explants of *Justicia gendarussa* Burm. f. The response for the induction of callus was more vigorous in the medium supplemented with 2, 4 – D or NAA in combination with BAP/Kn compared to the medium with IAA in combination with BAP/Kn. The callus induction in the medium supplemented with IAA + BAP/Kn was confined only to the cut ends of the explants, while the medium supplemented with 2, 4 – D/NAA + BAP induced morphologically better callus. The histological study of the root inducing callus of *J. gendarussa* showed the presence of numerous xylem tracheids arranged in the form of a ring with a central cavity indicating the tissue differentiation.

Key words: *Justicia gendarussa*, callus induction, root induction, histology

INTRODUCTION

Justicia gendarussa Burm. f. is an important medicinal plant belong to Acanthaceae and is traditionally used in the treatment of fever, rheumatism, carbuncles, diarrhea, pains in the head, ear, paralysis and bruises [1]. The pharmacological studies revealed the antioxidant, anti-arthritic, anti-inflammatory, analgesic, antifertility, anticancer, hepatoprotective and larvicidal properties [2-4]. The *in vitro* culturing of stem and leaf explants of *J. gendarussa* for the direct and indirect plant regeneration have been reported [5-10]. Bhagya and Chandrasheakr [9] reported the influence of different concentrations of auxins in the induction of callus from the stem and leaf explants of *J. gednarussa*.

The growth regulators show their influence on the *in vitro* morphogenesis of cultured plant tissues [11]. Salma *et al.* [12] studied the effect of different GR on the nodal explants of *Rauwolfia serpentina* L. and observed the optimum callus induction on MS medium supplemented with BAP + NAA at the concentrations of 0.5 + 2mg/l. The effect of different GR on *Cananga odorata* flower petals was studied by Nurazah *et al.* [13] and observed the induction of callus on MS nutrients with B5 vitamins and NAA + BAP (3 + 0.5mg/l). The study also showed the role of high concentration of NAA with the induction of pale, white and friable callus after 1-2 weeks of incubation. The effect of plant growth regulators on callus induction from different source and types of explants have been reported in *Catalpa bungei* [14], *Citrus jambhiri* Lush [15], *Amygdalus communis* [16] and rapeseed (*Brassica napus* L.) [17]. In the present study, the influence of combinations of auxins and cytokinins on callus induction and direct organogenesis has been studied.

MATERIALS AND METHODS

Plant materials and surface sterilization

The stem and leaf materials of *Justicia gendarussa* were collected from natural forests of Dakshina Kannada District, Karnataka, India. The surface sterilization of the stem and leaf samples were carried out as reported by Bhagya and Chandrashekar [9]. The collected materials were washed thoroughly under running tap water for 20-30min to remove the surface debris and treated with Bavistine for 30-45min. The explants were then sterilized with 70% alcohol (2min) and 0.1% $HgCl_2$ (8 min). After each step, the explants were washed using sterile distilled water.

Effect of combinations of auxin and cytokinin on stem and leaf explants

The sterile explants with ~5cm size were inoculated on to MS medium with varying concentrations of auxins in combination with cytokinins. The selected GR include 2, 4 – D, NAA, IAA, BAP and Kn at the concentrations of 0.1, 0.5, 1, 2, and 3mg/l. The cultures were incubated at 25±2°C with 16 hrs of photoperiod and 40.0±3.0µmol m^{-2} s^{-1} light intensity. The cultures were observed at the end of 25-30d of incubation for various details such as development of callus from mature or immature regions of the explants, nature of callus, color/pigmentation of the callus, initiation of roots and shoots.

Histology of root inducing explants

The explants showing root induction were fixed in Carnoy's 'B' fluid (Alcohol: Chloroform: Acetic acid

– 60:30:10) and processed through a series of graded alcohol from 70% to Ab alcohol keeping 24hr in each grade followed by alcohol:butanol (3:1, 1:1 and 1: 3) and butanol. The tissues were later embedded in paraffin wax and the sections were taken with a thickness of 10µm using Leica RM 2145 Microtome. The sections were stained using Toludine blue reagent.

STATISTICAL ANALYSIS

The data presented are the average of three replicates with 10 explants in each trial and expressed as Mean ± SD. The statistical analysis of all the data were carried out using SAS package (version 9.0) and the treatment means were compared using Duncun's Multiple Range Test (DMRT) at a level of 5% significance.

RESULTS

The stem and leaf explants of *J. gendarussa* cultured on medium supplemented with the combinations of auxin and cytokinin showed morphologically different types of callus irrespective of the concentrations of GR used in the study (Table 1).

Effect of 2, 4 – D + BAP/Kn on the stem and leaf explants

The medium supplemented with 2, 4 – D in combination with BAP/Kn induced a large mass of friable, creamish pink colored callus (Fig 1a & b).

Table 1. Effect of GR on callus, root and shoot induction from stem and leaf explants of *J. gendarussa*

GR	Stem				Leaf			
	Callus texture	**Callus color**	**Root induction**	**Shoot induction**	**Callus texture**	**Callus color**	**Root induction**	**Shoot induction**
2, 4 – D + BAP/ Kn	Friable	Creamish and pink	Nil	Nil	Friable	Creamish and pink	Nil	Nil
NAA + BAP/Kn	Compact	Yellow to dark green	Yes	Yes	Compact	Yellow to dark green	Yes	Nil
IAA + BAP/Kn	Compact	Yellow to light green	Yes	Yes	Compact	Yellow to light green	Yes	Nil

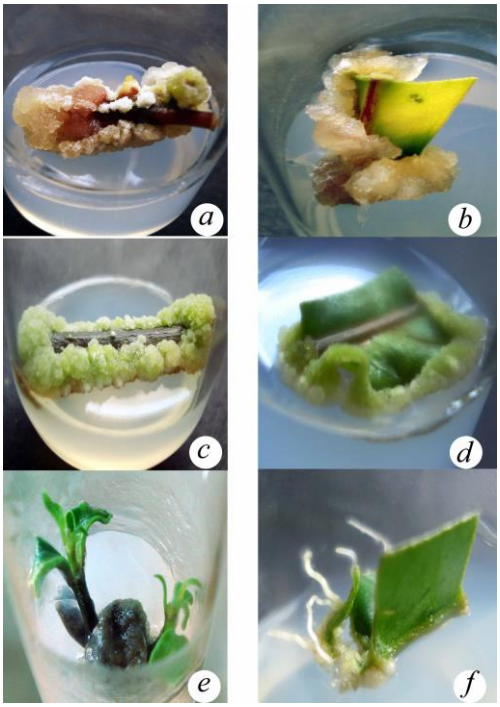

Fig. 1. a. Callus induction from stem explant on the medium with 2,4-D + BAP/Kn **b.** Callus induction from leaf explant on the medium with 2,4-D + BAP/Kn **c.** Callus induction from the stem explant on the medium with NAA + BAP/Kn **d.** Callus induction from leaf explant on medium with NAA + BAP/Kn **e.** Shoot induction from nodal explant on medium with NAA+ BAP **f.** Root induction from leaf explant on medium with NAA + BAP/Kn

The stem explants showed a highest number of callus induction (8.50 ± 0.70 explants) at 0.5 + 2mg/l concentrations of 2, 4 – D + BAP (Table 2). Similarly, when the medium supplemented with 2, 4 – D + Kn at 1 + 2mg/l concentrations showed a highest number of callus induction (9.00 ± 1.41 explants) (Table 2). The leaf explants of *J. gendarussa* showed a maximum callus induction (8.50 ± 0.71 explants) when cultured on medium supplemented with 2, 4 – D + BAP at the concentrations of 1 + 1.0/2.0mg/l (Table 2). A maximum callus induction (10.00 ± 0.00 explants) in the leaf explants were observed when cultured on medium supplemented with 2, 4 – D + Kn at the concentration 1 + 2mg/l (Table 2).

Effect of NAA + BAP/Kn on the stem and leaf explants

Medium fortified with NAA in combination with BAP/Kn induced compact, yellow to dark green colored callus (Fig 1c & d) and direct shoot organogenesis in the stem explants (Fig 1e) and also some of the culture tubes showed the induction of roots in both stem and leaf explants (Fig 1f, Table 3). The cultures close to the light were green in color and away from the light were more or less yellowish in color. The stem explants showed a maximum callus induction (9.50 ± 0.71 explants) on the medium supplemented with NAA + BAP at the concentrations of 0.5 + 3mg/l and 3 + 0.5mg/l (Table 3). The leaf explants showed a maximum callus induction (9.00 ± 0.00 explants) on the medium supplemented with NAA + BAP at the concentrations of 2 + 0.5mg/l (Table 3). The stem explants showed a highest number of 4.00 ± 0.00 explants for root induction in the medium supplemented with NAA + BAP at 3 + 1mg/l (Table 3). The nodal explants showed a highest mean number of 4.00 ± 0.00 explants for direct shoot induction in the medium supplemented with NAA + BAP at 2 + 1mg/l (Table 3). The leaf explants showed a significantly highest number of 4.50 ± 0.71 explants for root induction when cultured on medium supplemented with NAA + BAP at the concentrations of 2/3 + 0.1mg/l (Table 3).

Table 2. Effect of GR on callus induction from *J. gendarussa* stem and leaf explants: 2, 4 – D + BAP and 2, 4 – D + Kn

2, 4-D		Number of explants showing callus* (Mean±SD)				
		0.1	0.5	1.0	2.0	3.0
		BAP				
	Stem	4.50±0.71 $^{b-f}$	5.00±1.41 $^{b-e}$	5.00±1.41 $^{b-e}$	8.00±1.41 ^{a}	6.00±1.41^{b}
0.1	Leaf	4.50±0.70 $^{b-g}$	3.00±0.00 $^{b-g}$	5.00±1.41 $^{b-g}$	4.00±1.41 $^{b-g}$	7.50±0.71 ^{a}
	Stem	7.00±0.00 ^{a}	5.50±0.71^{b-d}	5.50±0.71 $^{b-d}$	8.50±0.70 ^{a}	6.50±0.71^{a}
0.5	Leaf	4.50±0.71 $^{b-g}$	6.00±0.00 ^{bc}	7.50±0.70 ^{a}	6.00±0.00 ^{bc}	7.00±1.41 ^{a}
	Stem	7.50±0.71 ^{a}	7.00±1.41^{a}	7.00±1.41^{a}	5.00±0.00 $^{b-e}$	5.00±1.41 $^{b-e}$
1.0	Leaf	6.00±0.00 ^{bc}	7.50±0.71^{a}	8.50±0.71 ^{a}	8.50±0.71 ^{a}	7.00±0.00 ^{a}
	Stem	5.50±0.70 $^{b-d}$	4.50±0.71 $^{b-f}$	6.00±1.41^{b}	5.50±0.70 $^{b-d}$	8.00±1.41 ^{a}
2.0	Leaf	3.50±0.70 $^{b-g}$	2.00±1.41 $^{b-g}$	5.50±0.71 $^{b-f}$	4.50±0.71 $^{b-g}$	3.00±1.41 $^{b-g}$
	Stem	7.00±0.00 ^{a}	5.50±0.70 $^{b-d}$	6.50±0.70 ^{a}	6.50±2.12 ^{a}	5.50±2.12 $^{b-d}$
3.0	Leaf	2.00±1.41 $^{b-g}$	3.50±0.70 $^{b-g}$	6.50±0.70 ^{bc}	7.00±0.00 ^{a}	8.50±0.70 ^{a}
	Stem	6.30±1.25	5.50±1.18	6.00±1.15	6.70±1.70	6.20±1.55
	Leaf	4.10±1.52	4.40±2.22	6.60±1.51	6.00±1.83	6.60±2.12
For Stem: SE/Plot = 1.14, CV (%) = 18.57; For Leaf: SE/Plot = 0.86, CV (%) = 15.53						
		Kn				
	Stem	8.00±0.00 ^{a}	4.00±1.41 $^{b-e}$	2.50±0.71 $^{b-e}$	1.50±0.71 $^{b-e}$	2.00±0.00 $^{b-e}$
0.1	Leaf	7.50±2.12 ^{bc}	4.50±0.71 $^{b-e}$	2.00±1.41 $^{b-e}$	2.00±0.00 $^{b-e}$	3.00±0.00 $^{b-e}$
	Stem	4.00±0.00 $^{b-e}$	2.00±1.41 $^{b-e}$	2.50±0.71 $^{b-e}$	2.00±0.00 $^{b-e}$	0.00±0.00 $^{b-e}$
0.5	Leaf	1.50±0.70 $^{b-e}$	2.00±1.41 $^{b-e}$	2.50±2.12 $^{b-e}$	2.50±0.70 $^{b-e}$	0.00±0.00 $^{b-e}$
	Stem	4.50±0.71 $^{b-e}$	6.50±0.70 ^{bc}	7.00±0.00 ^{b}	9.00±1.41 ^{a}	6.00±1.41 $^{b-d}$
1.0	Leaf	2.00±1.41 $^{b-e}$	7.00±1.41 $^{b-d}$	7.00±0.00 $^{b-d}$	10.00±0.00 ^{a}	9.00±0.00 ^{a}
	Stem	7.00±1.41 ^{b}	8.00±0.00 ^{a}	8.50±0.71 ^{a}	7.50±0.71 ^{a}	7.50±0.71 ^{a}
2.0	Leaf	9.50±0.71 ^{a}	7.50±0.71 ^{bc}	7.50±0.71 ^{bc}	6.50±0.70 $^{b-d}$	5.50±0.71 $^{b-e}$
	Stem	1.50±0.71 $^{b-e}$	3.50±0.71 $^{b-e}$	4.50±0.71 $^{b-e}$	6.00±1.41 $^{b-d}$	7.00±1.41 ^{b}
3.0	Leaf	6.00±0.00 $^{b-d}$	5.50±0.70 $^{b-e}$	7.00±1.41 $^{b-d}$	7.50±0.71 ^{bc}	7.00±1.41 $^{b-d}$
	Stem	5.00±2.49	4.80±2.39	5.00±2.58	5.20±3.22	4.50±3.21
	Leaf	5.30±3.40	5.30±2.21	5.20±2.74	5.70±3.23	4.90±3.35
For Stem: SE/Plot = 0.88, CV (%) = 18.02; For Leaf: SE/Plot = 1.02, CV (%) = 19.31						

* Values are the means of three experiments with ten explants each. Values with the different letters indicate significant difference at 5% level.

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Table 3. Effect of GR on callus induction from *J. gendarussa* stem and leaf explants: NAA + BAP and NAA + Kn

NAA		Number of explants showing callus* (Mean±SD)				
		0.1	0.5	1.0	2.0	3.0
		BAP				
	Stem	5.00±0.00 b-e	5.00±0.00 b-e	7.50±0.71 b-d	7.00±0.00 b-e	7.50±0.71 b-d
0.1	Leaf	7.00±0.00 b	5.50±0.70 b-f	8.00±0.00 a	7.50±0.71 a	7.00±1.41 b
	Stem	7.00±1.41 b-e	6.50±2.12 b-e	6.00±1.41 b-e	8.00±0.00 bc	9.50±0.71 a
0.5	Leaf	8.50±0.71 a	7.50±0.71 a	5.50±0.71 b-f	6.50±0.71 b-e	6.00±1.41 b-f
	Stem	9.00±0.00 a	8.00±1.41bc	7.00±1.41 b-e	7.00±0.00 b-e	8.00±1.41 bc
1.0	Leaf	8.00±0.00 a	5.00±0.00 b-f	6.50±0.71 b-e	6.00±1.41 b-f	5.00±1.41 b-f
	Stem	8.00±1.41bc	7.50±0.71b-d	7.00±1.41 b-e(40%S)	8.50±0.71 b	7.00±0.00 b-e
2.0	Leaf	8.50±0.71 a(45% R)	9.00±0.00 a	8.00±0.00 a	8.00±1.41 a	7.00±1.41 b
	Stem	7.00±1.41 b-e	9.50±0.71 a	7.00±1.41 b-e	7.50±0.71 b-d	7.00±0.00 b-e(40% R)
3.0	Leaf	8.00±0.00 a(45% R)	6.00±0.00 b-f	5.50±0.71 b-f	6.00±0.00 b-f	8.50±2.12 a
	Stem	7.20±1.61	7.30±1.83	6.90±1.10	7.60±0.70	7.80±1.14
	Leaf	8.00±0.67 #	6.60±1.58 $	6.70±1.25 $	6.80±1.14 $	6.70±1.70 $
For Stem: SE/Plot = 1.02, CV (%) = 13.86; For Leaf: SE/Plot=0.92, CV (%)=13.12						
		Kn				
	Stem	8.50±0.71 a	7.00±0.00 b	8.00±0.00 a	8.50±0.71a	6.00±1.41 bc
0.1	Leaf	6.33±3.79 b-g	9.50±0.71b	10.00±0.00 a	8.50±0.71 bc	6.50±0.70b-e
	Stem	7.50±2.12 a	5.50±0.71bc	4.00±1.41 bc	4.00±1.41 bc	4.50±0.71 bc
0.5	Leaf	8.50±0.70 bc	2.50±0.71 b-m	6.50±2.12 b-e	6.00±1.41 b-h	3.50±0.70 b-m
	Stem	5.50±0.71 bc	3.00±0.00 bc	2.50±0.71 bc	4.50±0.71 bc	5.50±2.12 bc
1.0	Leaf	4.50±2.12 b-l	5.00±2.83 b-j	2.50±0.71 b-m	1.00±0.00 b-m	2.00±1.41 b-m
	Stem	1.00±0.00 bc	4.50±2.12 bc(45%R)	8.00±1.41a	7.50±2.12a	4.50±2.12 bc(35% S)
2.0	Leaf	2.00±1.41 b-m	2.50±0.71 b-m	2.50±2.12 b-m	2.00±0.00 b-m	5.00±1.41 b-j
	Stem	5.50±0.71 bc(45%R)	6.00±1.41 bc	5.50±0.71 bc	3.50±0.71 bc	4.50±2.12 bc
3.0	Leaf	5.50±0.70 b-i(30%R)	4.50±0.71 b-l	4.50±0.70 b-l	3.50±0.71 b-m	3.00±1.41 b-m
	Stem	5.60±2.84	5.20±1.69	5.60±2.41	5.60±2.32	5.00±1.49
	Leaf	5.45±2.88	4.80±2.90	5.20±3.16	4.20±2.94	4.00±1.89
For Stem: SE/Plot = 1.29, CV (%) = 24.00; For Leaf: SE/Plot = 1.59, CV (%) = 33.62						

* Values are the means of three experiments with ten explants each. Where, R refers to the explants showing root induction in % and S refers to the explants showing shoot induction in %. Values with the different letters indicate significant difference at 5% level.

The stem explants showed a maximum callus induction (8.50 ± 0.71explants) when cultured on the medium supplemented with NAA + Kn at the concentration of 0.1 + 0.1/2mg/l (Table 3). When the leaf explants were cultured on medium supplemented with NAA + Kn at the concentration of 0.1 + 1mg/l induced significantly highest mean number of callus induction with 10.00 ± 0.00 explants (Table 3). The stem explants showed a highest root induction (4.50 ± 0.71 explants) when cultured on medium supplemented with NAA + Kn at the concentrations of 2 + 0.5 and 3 + 0.1mg/l (Table 3), while the nodal explants showed a significantly highest mean number of 3.50 ± 0.71 explants for direct shoot induction when cultured on medium supplemented with NAA + Kn at 2 + 3mg/l concentration (Table 3). The leaf explants showed a significantly highest mean number of root induction from 3.00 ± 1.41 explants when cultured on medium supplemented with NAA + Kn at the concentrations of 3 + 1mg/l (Table 3).

Effect of IAA + BAP/Kn on the stem and leaf explants

IAA in combination with BAP/Kn showed compact and yellow to light green colored callus (Fig 2a & b). The stem explants showed direct organogenesis and root induction in the medium supplemented with IAA in combination with BAP/Kn (Fig 2c) whereas, the leaf explants showed callus induction and root induction. The stem explants showed a highest callus induction (8.50 ± 2.12 explants) when cultured on medium supplemented with IAA + BAP at the concentrations of 0.1 + 2mg/l (Table 4). The leaf explants showed a highest callus induction (8.50 ± 0.71 explants) when cultured on medium supplemented with IAA + BAP at the concentration of 3 + 0.1mg/l concentration (Table 4).

The stem explants showed a highest mean number of 4.50 ± 0.71 explants for root induction in the medium supplemented with IAA + BAP at the concentrations of 3 + 2mg/l (Table 4). The nodal explants cultured on medium with IAA + BAP showed direct shoot induction with mean number of 3.50 ± 0.71 explants at the concentration of 0.1 + 2mg/l (Table 4). The

stem explants cultured on medium supplemented with IAA + Kn at the concentration of 1 + 1mg/l induced a significantly highest mean number of 2.00 ± 0.00 explants for root induction (Table 4). The nodal explants cultured on medium supplemented with IAA + Kn at the concentration of 0.1 + 1mg/l showed significantly highest response (4.50 ± 2.12) for direct shoot induction (Table 4).

The response of the explants was more vigorous in the medium supplemented with 2, 4 – D or NAA in combination with BAP/Kn compared to the medium with other GR. The callus induction in the medium supplemented with IAA + BAP/Kn was confined only to the cut ends of the explants, while the medium supplemented with NAA + BAP at the concentration of 1 + 0.1mg/l induced morphologically better callus (Table 1).

Histology of root inducing callus

The histological study of the root inducing callus of *J. gendarussa* showed the presence of numerous xylem tracheids arranged in the form of a ring with a central cavity indicating the tissue differentiation (Fig 2d). In these sections, many xylem cells are arranged from the central pith region towards the periphery as exarch arrangement which developed into actinostele with the addition of phloem cells at the outer region.

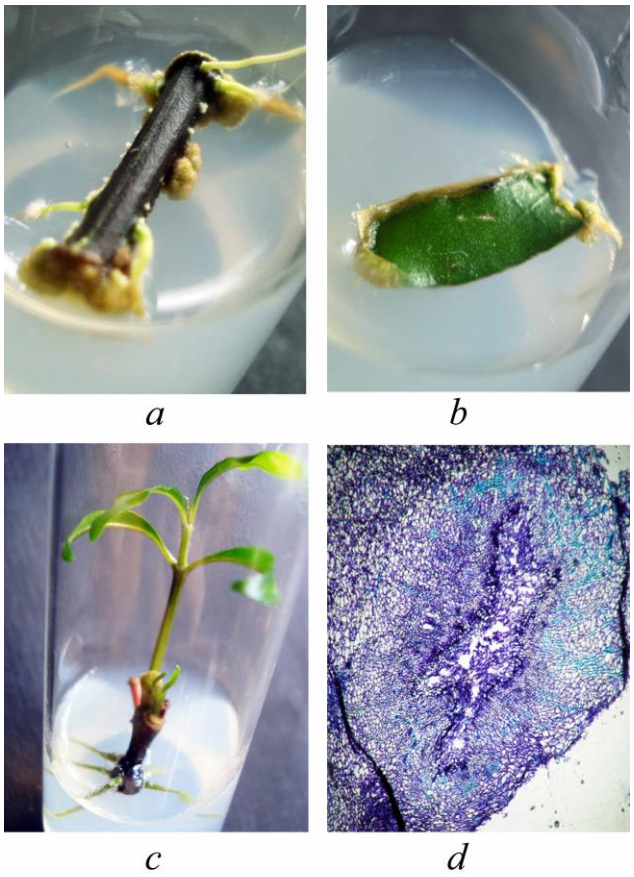

Fig. 2. a. Callus and root induction from stem explant on medium with IAA + BAP/Kn **b.** Callus and root induction from leaf explant on medium with IAA + BAP/Kn **c.** Shoot and root induction from nodal explant on medium with IAA + BAP/Kn **d.** Histology of root inducing callus

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Table 4. Effect of GR on callus induction from *J. gendarussa* stem and leaf explants: IAA + BAP and IAA + Kn

IAA		Number of explants showing callus* (Mean±SD)				
		0.1	**0.5**	**1**	**2**	**3**
				BAP		
	Stem	0.00±0.00 $^{b-e}$	6.00±1.41 ^{bc}	4.00±1.41 $^{b-e}$	8.50±2.12 ^{a}(35%S)	7.50±3.54 ^{a}
0.1	Leaf	2.50±0.71 ^{bcd}	3.00±1.41 ^{bcd}	2.50±0.71 ^{bcd}	2.50±0.71 ^{bcd}	2.00±1.41 ^{bcd}
	Stem	3.50±0.71 $^{b-e}$	6.00±1.41 ^{bc}	5.50±0.71 ^{bc}	3.50±0.71 $^{b-e}$	3.00±0.00 $^{b-e}$
0.5	Leaf	3.00±0.00 ^{bcd}	3.50±0.71 ^{bcd}	1.50±0.71 ^{bcd}	2.50±0.71 ^{bcd}	5.00±1.41 ^{bcd}
	Stem	3.00±0.00 $^{b-e}$	3.50±0.71 $^{b-e}$	4.00±0.00 $^{b-e}$	2.50±0.71 $^{b-e}$	3.00±1.41 $^{b-e}$
1	Leaf	6.00±0.00 ^{bc}	5.50±0.71^{bc}	3.50±0.71 ^{bcd}	3.00±1.41 ^{bcd}	3.50±2.12 ^{bcd}
	Stem	2.00±0.00 $^{b-e}$	3.50±0.71 $^{b-e}$	5.50±0.71 ^{bc}	3.50±0.71 $^{b-e}$	4.00±1.41 $^{b-e}$
2	Leaf	3.00±0.00 ^{bcd}	5.00±0.00 ^{bcd}	6.00±0.00 ^{bc}	8.00±0.00 ^{a}	6.50±2.12 ^{bc}
	Stem	6.50±0.71 ^{b}(45%R)	5.00±1.41 $^{b-d}$	5.00±0.00 $^{b-d}$	5.00±1.41 $^{b-d}$	6.00±0.00 ^{bc}
3	Leaf	8.50±0.71 ^{a}	6.00±1.41 ^{bc}	5.50±0.71 ^{bc}	3.50±0.71 ^{bcd}	6.50±2.12 ^{bc}
	Stem	3.00±2.26 $^{\$}$	4.80±1.48 $^{\#}$	4.80±0.92 $^{\#}$	4.60±2.41 $^{\#}$	4.70±2.31 $^{\#}$
	Leaf	4.60±2.46	4.60±1.43	3.80±1.87	3.90±2.33	4.70±2.31
For Stem: SE/Plot = 1.19, CV (%) = 27.21; For Leaf: SE/Plot = 1.11, CV (%) = 25.78						
				Kn		
	Stem	0.00±0.00 (20% R)	5.00±2.83	6.50±3.54(45% S)	5.50±0.71	4.00±1.41
0.1	Leaf	5.00±1.41	4.50±2.12	8.00±1.41	5.00±2.83	4.00±2.83
	Stem	2.00±0.00	4.00±1.41	5.00±1.41	6.50±0.71	3.00±0.00
0.5	Leaf	2.00±1.41	3.00±1.41	4.00±1.41	4.50±2.12	3.00±1.41
	Stem	4.00±1.41	5.00±1.41	6.50±0.71	3.50±2.12	5.00±0.00
1	Leaf	3.00±2.83	4.50±0.70	6.50±0.71	6.00±0.00	7.00±0.00
	Stem	3.50±2.12	5.00±1.41	4.50±0.71	6.00±1.41	5.50±2.12
2	Leaf	7.50±0.71	8.50±0.70	5.50±0.71	6.00±1.41	5.50±2.12
	Stem	6.00±2.83	5.00±0.00	3.00±1.41	5.00±2.83	3.50±0.71
3	Leaf	5.00±1.41	6.00±1.41	7.00±1.41	6.50±0.71	8.00±0.00
	Stem	3.10±2.47 $^{\$}$	4.80±1.32 $^{\#}$	5.10±1.97 $^{\#}$	5.30±1.70 $^{\#}$	4.20±1.32 $^{\$}$
	Leaf	4.50±2.37	5.30±2.21	6.20±1.69	5.60±1.51	5.50±2.32
For Stem: SE/Plot = 1.66, CV (%) = 36.78; For Leaf: SE/Plot = 1.56, CV (%) = 28.70						

* Values are the means of three experiments with ten explants each. Where, R refers to the explants showing root induction in % and S refers to the explants showing shoot induction in %. Values with the different letters indicate significant difference at 5% level

DISCUSSION

The callus obtained from the stem and leaf explants of *J. gendarussa* in the medium supplemented with 2, 4 - D in combination with cytokinin showed the formation of friable callus with pink color while, the callus obtained in the medium with NAA/IAA alone or with cytokinin was cream, light yellow and green colored. In *Pulsatilla koreana* Nakai, the medium supplemented with Kn produced soft, light green callus after two weeks of incubation [18]. The callus obtained from *Ruta graveolens* in the medium supplemented with NAA was compact and yellow colored which later converted to greenish yellow upon transfer to medium containing NAA + BAP [19]. The callus obtained from the nodal and leaf explants of *Phyllanthus amarus* was dark green, nodular and hard on medium with BAP and TDZ, whereas loose and pale green on medium with Kn [20]. Similar results were observed in the present study also. The development of pink colored callus in the medium supplemented with 2, 4 – D might be due to the production of anthocyanins in the callus. The formation of green colored calli of *Solanum tuberosum* in the medium supplemented with NAA and BAP was also reported by Shirin *et al.* [21] and similar result was observed in the present study as well. Based on the results obtained in different plant species and from the present study, it is clear that different growth regulators have varying effects on the nature of callus its texture and color. The combination of auxin with cytokinin induced rooting in the protoplast derived calli of *Pyrus communis* L. [22], stem/ leaf derived callus of *Heliotropicum indicum L.* [23] and *Brynopsis laciniosa* L. [24]. Similarly, in the present study also some of the cultures of *J. gendarussa* stem and leaf explants maintained at higher concentrations of NAA or IAA in combination with BAP or Kn led to the induction of roots. The callus obtained from the nodal and leaf explants of *Phyllanthus amarus* on MS medium supplemented with IAA and NAA was off-white and friable and produced numerous roots whereas, callus produced on the media fortified with 2, 4 - D was yellowish and loose without any root formation [20]. The induction of direct shoots from the nodal explants was reported in the members of Acanthaceae viz. *Adhatoda vasica* Nees [25], *Beloperone plumbaginifolia* (Jacq.) [26]. The direct shoot proliferation from the nodal explants when cultured on medium supplemented with combinations of NAA

with BAP/Kn was also reported in *J. gendarussa* by Johnson *et al.* [5], Agastian *et al.* [6], Bushrabi *et al.* [7] and Janarthanam and Sumathi [27]. Almost similar results were observed in the present study also.

The histology of root inducing calli of *J. gendarussa* showed the presence of numerous xylem tracheids. The cultures showing rhizogenesis at different concentrations of GR were reported in *Vitis rupestris* du Lot [28], *Vigna radiata* W. [29] and opium poppy [30]. In the present study also many xylem cells were arranged from the central pith region towards the periphery as exarch arrangement, probably, with the addition of phloem cells at the outer region, this may develop into actinostele.

REFERENCE

[1] Jain S.K. (1991) Dictionary of Indian folk medicine and ethnobotany, A reference manual of man-plant relationships and ethnobotanists, Deep publications, New Delhi, India, pp: 110

[2] Paval J., S.K. Kaitheri, B.K. Potu, R.S. Kumar, S.N. Narayanan, S. Moorkoth (2009) Anti-Arthritic Potential of the Plant *Justicia gendarussa* Burm F. Clinics (Sao Paulo). 64(4): 357–362

[3] Ratnasooriya W.D., S.A. Deraniyagala, D.C. Dehigaspitiya (2007) Antinociceptive activity and toxicological study of aqueous leaf extract of *Justicia gendarussa* Burm. F. in rats. - Pharmacog Mag. 3: 145-155

[4] Senthilkumar N., P. Varma,Gurusubramanian (2009) Larvicidal and adulticidal activities of some medicinal plants against the malarial vector *Anopheles stephensi* (Liston). – Parasitol. Res., 104: 237-244

[5] Johnson M., V.S. Manickam, Das, Sonali, Y. Nikhat, N. Andal (2004) *In vitro* multiplication of two economically important and endangered medicinal plants– *Justicia gendarussa* Burm and *Adenia hondala* (Gaertn) De Wilde. – Malays. J. Sci., 23: 49-53

[6] Agastian P., L. Williams, S. Ignacimuthu (2006) *In vitro* propagation of *Justicia gendarussa* Burm. f.– A medicinal plant. – Ind. J. Biotechnol., 5: 249-251

[7] Bushrabi N.K., P. Drisyadas, S. Benjamin, P.V. Madhusoodanan (2008) *In vitro* plant development of *Justicia gendarussa* -J Trop. Med. Plants. 9: 59-63

[8] Thomas T.D., H. Yoichiro (2010) *In vitro* propagation for the conservation of a rare medicinal plant *Justicia gendarussa* Burm. f. by nodal explants and shoot regeneration from callus. - Acta Physiologiae Plantarum, 32: 943-950

[9] Bhagya N., K.R. Chandrashekar (2010) Effect of auxin concentration on Callus induction from *Justicia gendarussa* L. stem and leaf explants. - International Journal of Biosciences and technology, 3: 27-35

[10] Bhagya N., K.R. Chandrashekar, A. Karun, U. Bhavyashree (2012) Plantlet regeneration through indirect shoot organogenesis and somatic embryogenesis in *Justicia gendarussa* Burm. f., a medicinal plant. – J. Plant Biochem. Biotechnol., DOI. 10.1007/s13562-012-0177-3

[11] Gubis J., Z. Lajchova, J. Farago, Z. Jurekova (2004) Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.), Biologia (Bratislava)., 59: 405-408

[12] Salma U., M.S.M. Rahman, S. Islam, N. Haque, T.A. Jubair, A.K.M.F. Haque, I.J. Mukti (2008) The influence of different hormone concentration and combination on callus induction and regeneration of *Rauwolfia serpentina* L. Benth, Pakistan J. Biol. Sci., 11: 1638-1641

[13] Nurazah Z., M. Radzali, A. Syahida, M. Maziah (2009) Effect of plant growth regulators on callus induction from *Cananga odorata* flower petal explant, Afr J Biotechnol., 8: 2740-2743

[14] Juan L., W. Lihua, L. Jing, W. Junhui (2010) Effect of different plant growth regulators on callus induction in *Catalpa bungei*, Afr. J. Agr. Res.,5(19): 2699-2704

[15] Savita, G.S. Vijay, Virk, A. Nagpal (2010) Effect of explant type and different plant growth regulators on callus induction and plantlet regeneration in *Citrus jambhiri* Lush Environ. We, Int. J. Sci. Tech., 5: 97-106

[16] Sharifmoghaddam N., A. Safarnejad, S.M. Tabatabaei (2011) The effect of plant growth regulators on callus induction and regeneration of *Amygdalus communis*, Not. Sci. Biol., 3(3):97-100

[17] Afshari R.T., R. Angoshtari, S. Kalantari (2011) Effects of light and different plant growth regulators on induction of callus growth in rapeseed (*Brassica napus L.*) genotypes, POJ 4(2):60-67

[18] Lin G-Z., X-M. Zhao, S-K. Hong, Y-J. Lian (2011) Somatic embryogenesis and shoot organogenesis in the medicinal plant *Pulsatilla koreana* Nakai. - Plant cell tissue and organ cult., 106: 93-103

[19] Tejavathi D.H., B.L. Manjula (2010) Studies on organogenesis from nodal explant of *Ruta graveolens* L. - The Bioscan., 5: 455-459

[20] Nitnaware K.M., D.G. Naik, T.D. Nikam (2011). Thidiazuron-induced shoot organogenesis and production of hepatoprotective lignan phyllanthin and hypophyllanthin in *Phyllanthus amarus*, Plant Cell Tiss Organ Cult., 104: 101–110

[21] Shirin F., M. Hossain, M.F. Kabir, M. Roy, S.R. Sarker (2007). Callus induction and plant regeneration from internodal and leaf explants of four potato (*Solanum tuberosum* L.) cultivers, World J Agricultral Sci., 3: 01-06

[22] Ochatt S.J., J.B. Power (1988) Plant regeneration from mesophyll protoplasts of Williams' Bon Chretien (syn. Bartlett) pear (*Pyrus communis* L.), Plant Cell Rep., 7: 587-589

[23] Bagadekar A.N., M. Jayaraj (2011) *In Vitro* flowering of *Heliotropium indicum*, L.-An important medicinal herb, Asian J Exp Biol Sci., 2: 2011: 90-95

[24] Caroline V.J.E., B. Mallaiah (2011) *In–Vitro* mutagenesis in an endangered medicinal Cucurbit *Bryonopsis Laciniosa* (L.) Naud., Int J Pharm Bio Sci., 2: 97-75

[25] Khalekuzzaman M., M.S. Rahman, M.H. Rashid, M.S. Hossain (2008) High frequency *in vitro* propagation of *Adhatoda vasica* Nees through shoot tip and nodal explants culture, J Biosci., 16: 35-39

[26] Shameer M.C., V.P. Saeeda, P.V. Madhusoodhanan, S. Benjamin (2009) Direct organogenesis and somatic embryogenesis in *Beloperone plumbaginifolia* (Jacq.) Nees, Indian J Biotechnol., 8: 132-135

[27] Janarthanam 1.B., E. Sumathi (2010) *In vitro* regeneration of *Justicia gendarussa* Burm. f., Libyan Agriculture. - Research Center Journal International, 1: 284-287

[28] Altamura M.M., A. Cersosimo, C. Majoli, M. Crespan (1992) Histological study of embryogenesis and organogenesis from anthers of *Vitis rupestris* du Lot cultured *in vitro*, Protoplasma, 171: 134-141

[29] Park J-B., K-B. Lee, S. Lee (2002) Histological study of callus formation and root regeneration from mung bean (*Vigna radiata* W.), J Plant Biol., 45: 170-176

[30] Kaseem M.A., A. Jacquin (2001) Somatic embryogenesis, rhizogenesis and morphinan alkaloids production in two species of opium poppy, J Biomed Biotechnol., 1: 70-78